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Directed evolution of toluene dioxygenase from Pseudomonas putida for improved selectivity toward cis-indandiol during indene bioconversion.

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Toluene dioxygenase (TDO) from Pseudomonas putida F1 converts indene to a mixture of cis-indandiol (racemic), 1-indenol, and 1-indanone. The desired product, cis-(1S,2R)-indandiol, is a potential key intermediate in the chemical synthesis of indinavir sulfate (Crixivan), Merck's HIV-1 protease inhibitor for the treatment of AIDS. To reduce the undesirable byproducts 1-indenol and 1-indanone formed during indene bioconversion, the recombinant TDO expressed in Escherichia coli was evolved by directed evolution using the error-prone polymerase chain reaction (epPCR) method. High-throughput fluorometric and spectrophotometric assays were developed for rapid screening of the mutant libraries in a 96-well format. Mutants with reduced 1-indenol by-product formation were identified, and the individual indene bioconversion product profiles of the selected mutants were confirmed by HPLC. Changes in the amino acid sequence of the mutant enzymes were identified by analyzing the nucleotide sequence of the genes. A mutant with the most desirable product profile from each library, defined as the most reduced 1-indenol concentration and with the highest cis-(1S,2R)-indandiol enantiomeric excess, was used to perform each subsequent round of mutagenesis. After three rounds of mutagenesis and screening, mutant 1C4-3G was identified to have a threefold reduction in 1-indenol formation over the wild type (20% vs 60% of total products) and a 40% increase of product (cis-indandiol) yield.

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